

## CLAIMS

1. A method for determining the presence or concentration of an analyte, comprising the steps of:

- a) exposing a fluorescent reference molecule and a  
5 fluorescent sensing molecule to a radiation source;
- b) measuring a first level of anisotropy of the combined fluorescence emitted by said molecules;
- c) exposing said sensing molecule to an analyte, wherein said analyte is capable of changing the intensity  
10 of the fluorescence emitted by the sensing molecule;
- d) measuring a second level of anisotropy of the combined fluorescence emitted by said molecules after exposure of the sensing molecule to said analyte; and
- e) correlating a change in said second level of  
15 anisotropy with the presence or concentration of said analyte.

2. The method of claim 1, wherein the fluorescent reference molecule is a long-lifetime metal-ligand  
20 complex.

3. The method of claim 1, wherein the fluorescent reference molecule displays an anisotropy near zero.

25 4. The method of claim 1, wherein the fluorescent reference molecule displays an anisotropy near one.

5. The method of claim 4, wherein the fluorescent reference molecule is embedded in a film.  
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6. The method of claim 5, wherein the film is a stretched polymer film.

7. The method of claim 1, wherein the analyte is  
35 selected from the group consisting of an ion, oxygen,

protein, lipoprotein, glycoprotein, peptide, nucleic acid, polysaccharide, lipopolysaccharide, lipid, fatty acid, cellular metabolite, hormone, pharmacological agent, antibody, and a sugar.

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8. The method of claim 1, wherein the method determines pH.

9. The method of claim 8, wherein the fluorescent sensing molecule is a pH sensitive fluorescein.

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10. The method of claim 9, wherein the fluorescent reference molecule is pyridine 2 in a stretched film.

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11. The method of claim 1, wherein the reference and sensing molecules are distinct molecules having the same structure, and wherein the reference molecule is not exposed to the analyte.

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12. The method of claim 1, wherein the reference molecule is exposed to the analyte only if such exposure is not capable of changing the intensity of the fluorescence emitted by the reference molecule.

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13. A device for determining the presence or concentration of an analyte, which comprises:

a) means for exposing a fluorescent reference molecule and a fluorescent sensing molecule to a radiation source;

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b) means for measuring the anisotropy of the combined fluorescence emitted by said molecules;

c) means for exposing said sensing molecule to an analyte;

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d) optionally means for correlating a change in said level of anisotropy from before to after exposure to

e) optionally a radiation source capable of causing  
said reference and sensing molecules to emit  
5 fluorescence.

Group 1 ①-12 method. can be measured using <sup>performed</sup> electron microscopy. as

Group 2 ⑬-14 device